Variations in the α_s-Casein Fraction of Individual Cow's Milk

We have investigated variations in the α_s-casein fraction[1], the calcium-sensitive component of α-casein and a major protein fraction of the casein family. Recently, Aschaffenburg[2] demonstrated the heterogeneity of β-casein and also noted the presence of γ-casein variants. The existence of 5 out of 6 possible phenotypes for β-casein was shown by paper electrophoresis in a citrate–phosphate–urea buffer at *p*H 7·15. The evidence presented suggested that β-casein polymorphism may be restricted to Channel Island breeds. Heterogeneity of α_s-casein fraction was not noted.

The components of the α-casein complex are not conveniently observed by conventional electrophoretic methods. However, the use of starch-gel-urea electrophoresis at *p*H 8·6 has proved to be an extraordinarily good method for separation of casein components. Using this technique, we investigated the homogeneity of the α_s-casein fraction. For determination of molecular parameters and structural features of this protein, assurance of its homogeneity is imperative. In addition, the detection of heterogeneity in α_s-casein would be important since the heterogeneity could possibly be related to the physical instability of the calcium caseinate complex in milk.

Individual bovine milks (Holstein, Brown Swiss × Holstein, and Ayrshire × Holstein) were selected for examination. They were obtained from the U.S. Department of Agriculture herd at Beltsville, Maryland. To prepare the samples, gravity-separated milk was precipitated at *p*H 4·6 and 22°–24° C. The precipitate was washed four times with water and ethanol, twice with acetone and twice with ethyl ether. Starch-gel-urea electrophoresis, as described by Wake and Baldwin[3], was run on casein solutions containing 7 mg casein per millilitre of buffer-urea solution. Approximately 5 V cm^{-1} was applied to the gel for 17 h, after which it was developed with amido black dye.

On examination of caseins from 93 individual cows by starch-gel-urea electrophoresis, patterns identical to pooled-milk caseins were usually obtained. However, in six cases an additional band appeared in the region ascribed to calcium-sensitive α-casein (α_s). The six individuals were all daughters of one sire, an

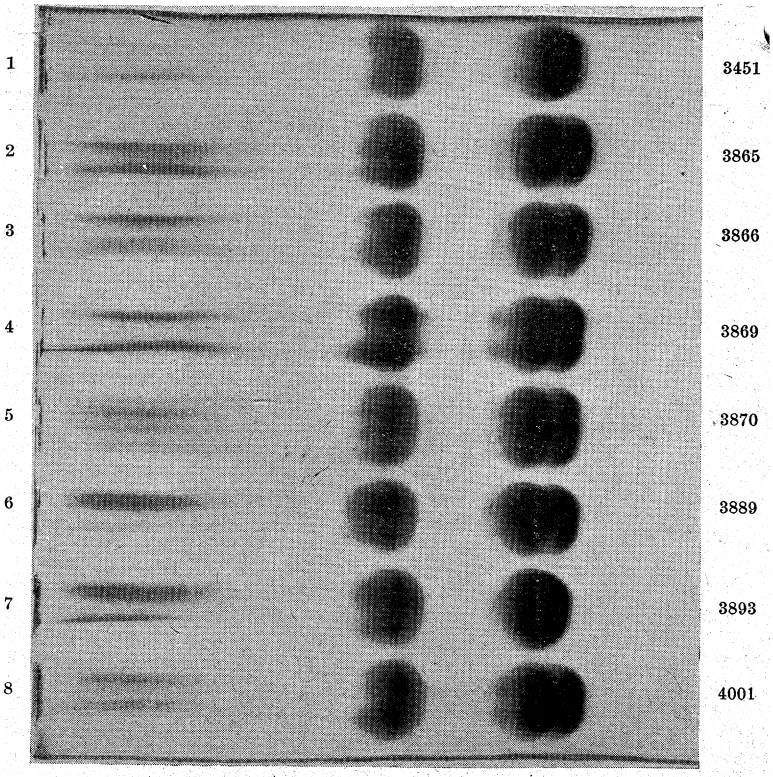

Fig. 1. Starch-gel-urea electrophoresis patterns of casein from individual cows

observation which suggested a genetic basis for the heterogeneity.

Fig. 1 shows starch-gel-urea electrophoresis patterns of individual caseins, the larger numbers (3451, 3865, etc.) referring to the animal number. Patterns 1 and 7 are like patterns obtained with pooled whole casein whereas the remainder of the patterns show a double α_s band referred to as α_s-A and α_s-B in order of decreasing mobility. The use of A and B further denotes genetic nomenclature of the α_s-caseins. All other caseins studied show α_s-B casein, but α_s-A has not been observed alone to date. On mixing equal quantities of α_s-B and α_s-A/B caseins and examining the pattern on starch-gel-urea electrophoresis, a pattern typical of α_s-A/B is obtained except that α_s-A is diminished in concentration. Little question exists that α_s-B from α_s-A/B and homozygous α_s-B are identical, but isolation and characterization will be the only unequivocal proof.

In regard to the occurrence of the α_s-A/B split over the course of lactation, we have observed with cow number 4001 that the heterogeneity occurred throughout a complete single lactation and into the next. Other animals (both α_s-A/B and α_s-B) have been examined within the same lactation with identical results.

The α_s-A/B heterogeneity is detectable in veronal buffer at pH 8·6, $\Gamma/2 = 0{\cdot}10$, in free boundary electrophoresis, and a bimodal schlieren pattern was observable in the ultracentrifuge at pH 7·0, $\Gamma/2 = 0{\cdot}20$ in phosphate buffer. Urea fractionation[4] yields both α_s-A/B in the 4·7 M insoluble fraction. Subsequent treatment of α_s-A/B with calcium chloride reveals that both are calcium-sensitive. In addition, α_s-A/B caseins are eluted together from DEAE-cellulose-urea chromatographic columns[5].

As already mentioned, the double α_s band has been observed only in caseins from daughters of a single sire (*P*-17). Of 14 daughters of this sire in the Beltsville herd, six showed the heterogeneity. These daughters are coded as 3865, 3866, 3869, 3870, 3889 and 4001 (all Holstein). At this point of the investigation little can be said of the genetic nature of the occurrence of α_s-A/B casein. However, the examination at this time is centring around studying daughter–dam pairs of the *P*-17 sire. In addition, we are seeking the possible occurrence of cows producing only α_s-A casein.

Note added in proof. Since the preparation of this manuscript an additional variant (α_s-C) has been discovered with the co-operation of Dr. R. Aschaffenburg, Reading, England. This discovery does not alter the acceptable nomenclature of α_s-A and α_s-B, and a detailed report of its occurrence is in preparation.

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[1] Waugh, D. F., and von Hippel, P. H., *J. Amer. Chem. Soc.*, **78**, 4576 (1956).

[2] Aschaffenburg, R., *Nature*, **192**, 431 (1961).

[3] Wake, R. G., and Baldwin, R. L., *Biochim. et Biophys. Acta*, **47**, 225 (1961).

[3] Hipp, N. J., Groves, M. L., Custer, J. H., and McKeekin, T. L., *J. Dairy Sci.*, **35**, 272 (1952).

[5] Ribadeau Dumas, B., *Biochim. et Biophys. Acta*, **54**, 400 (1961).

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